



Bettridge, CM, Kenworthy, SP, Butynski, TM, De Jong, YA and De Kort, SR ORCID logoORCID: <https://orcid.org/0000-0001-8588-5706> (2019) Vocal Repertoire and Intraspecific Variation within Two Loud Calls of the Small-Eared Greater Galago (*Otolemur garnettii*) in Tanzania and Kenya. *Folia Primatologica: international journal of primatology*, 90 (5). pp. 319-335. ISSN 0015-5713

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Version: Accepted Version

Publisher: Karger Publishers

DOI: <https://doi.org/10.1159/000500260>

Please cite the published version

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Vocal repertoire and intraspecific variation within long-distance calls of the small-eared
greater galago (*Otolemur garnettii*) in Tanzania and Kenya

Caroline M. Bettridge^a, Simon P. Kenworthy^a, Thomas M. Butynski^b, Yvonne A. de Jong^b,
and Selvino R. de Kort^a

^a Division of Biology & Conservation Ecology, John Dalton East, Manchester Metropolitan
University, Manchester, United Kingdom.

^b Eastern Africa Primate Diversity and Conservation Program, P. O. Box 149, Nanyuki, Kenya.

Running head: Loud calls of *Otolemur garnettii*

Word count: 4793

Corresponding author: Caroline Bettridge, Division of Biology & Conservation Ecology, John Dalton
East, Manchester Metropolitan University, Manchester, UK, M1 5GD, email:
c.bettridge@mmu.ac.uk. Tel: 0161 247 1198.

Key words: Advertisement call; Alarm call; Bioacoustics; Bushbaby; Communication;
Nocturnal primates; Population differences; Vocalisations

Abstract

Vocal repertoires and call structure can provide insights into the behaviour and evolution of species, as well as aid in taxonomic classification. Nocturnal primates have large vocal repertoires. This suggests that acoustic communication plays an important role in their life histories. Little is known about the behavioural context or the intraspecific variation of their vocalisations. We used autonomous recording units (ARUs) and manual recorders to investigate the vocal behaviour of the small-eared greater galago (*Otolemur garnettii*) in Kenya and Tanzania. We describe the vocal repertoire and temporal calling patterns of two subspecies; *O. g. panganiensis* and *O. g. kikuyuensis*. We found considerable intraspecific structural differences in the long-distance calls. These are congruent with the current subspecies classification. The differences in vocalisations between populations are not consistent with the ‘Acoustic Adaptation Hypothesis’, rather they are likely a result of geographic variation due to isolation caused by vegetational barriers in southern Kenya.

Introduction

Detailed knowledge of vocal repertoires may provide insight into the evolution of communication systems [Maynard Smith and Harper, 2003]. The ‘Social Complexity Hypothesis for Communication’ [Bradbury and Vehrencamp, 2001; Freeberg et al., 2012; Fischer et al., 2017] proposes that species living in more complex social environments have evolved more complex vocal communication systems. The idea that social complexity drives vocal complexity derives support from the positive relationship in non-human primates between vocal repertoire size and group size, and with time spent grooming [McComb and Semple, 2005]. For example, red-capped mangabeys (*Cercocebus torquatus*), which live in large and relatively despotic multimale-multifemale groups with frequent interactions, have a more complex vocal repertoire compared to De Brazza's monkeys (*Cercopithecus neglectus*), which live in small family groups [Bouchet et al., 2013].

Knowledge of particular types of vocalisations can contribute to our understanding of behaviour, evolution and taxonomy. Spectral parameters of calls reflect requirements for transmission in different habitats, as well as constraints imposed by body size [Masters, 1991]. The ‘Acoustic Adaptation Hypothesis’ relates acoustic variables to habitat features (e.g. density of vegetation) [Ey and Fischer, 2009], yet primate vocalisations are particularly resilient to modification by the immediate environment [Doyle, 1978]. Within a species’

vocal repertoire, close-range vocalisations are predicted to be more variable (i.e. less distinct) than long-range vocalisations (often referred to as ‘loud calls’ or ‘advertisement calls’) [Marler, 1967]. Among primates, loud calls are indicators of taxonomic delineation and many African monkeys can be readily distinguished on the basis of their loud calls [Struhsaker, 1970; Waser, 1982; Jones et al., 2005].

Intraspecific geographic variation in calls can result from differences in habitat, mechanisms of social learning, and genetic isolation. Geographic variation in calls is common in songbirds, who learn their vocalisations from conspecifics. This variation may be the result of inaccurate copying [Slater, 1989; Podos and Warren, 2007]. Less is known about geographic variation in primate vocalisations, although orangutans (*Pongo* spp.) [Delgado, 2007] and savanna monkeys (*Chlorocebus* spp.) [Price et al., 2014] show differences in temporal and spectral call structure among populations. Intraspecific differences in call structure also occur among populations of robust chimpanzees (*Pan troglodytes*) [Mitani et al., 1999; Crockford et al., 2004], silvery gibbons (*Hylobates moloch*) [Dallmann and Geissmann, 2001], and pygmy marmosets (*Callithrix pygmaea*) [De La Torre and Snowdon, 2009]. In strepsirhines, comparison of the spectral parameters of the loud call is the most useful diagnostic tool for subspecies identification [Zimmermann, 2012].

Non-human primates are historically described as non-learners with respect to the development of their vocal system. Evidence is accumulating, however, that acoustic plasticity is present in non-human primates and that it consists of subtle acoustic changes on top of innately determined call structures [Roian Egnor and Hauser, 2004]. This implies that spectral and temporal parameters in the vocalisations of primates could differ among populations as a result of social learning, adaptation to local habitats, or drift.

Vocalisations can convey information about the caller’s identity [Owren et al., 1997; Herbinger et al., 2009], size [Reby et al., 2005], physical condition [Benítez et al., 2016], motivation [Silk et al., 2000; Manser, 2001], and about external events such as predator presence or type [Seyfarth et al., 1980; Zuberbühler, 2001]. Some call types, such as those indicating presence of a predator, are predicted to be less variable than those more strongly influenced by sexual selection, and containing information about the caller’s identity and/or quality [Butynski et al., 1992; Irwin et al., 2008]. Geographic variation in vocal behavior has

been described in a wide range of species, but generally not in alarm calls [Zuberbühler 2009].

Galagos (Galagidae) have large, diverse, species-specific vocal repertoires. Their vocalisations have contributed to species delineation (e.g. southern lesser galago (*Galago moholi*), northern lesser galago (*G. senegalensis*), Somali galago (*G. gallarum*), Angolan dwarf galago (*Galagoides kumbirensis*) [Zimmermann et al., 1988; Zimmermann, 1995; Butynski and De Jong, 2004, Svensson et al., 2017]. There has been no attempt, however, to explore variation in call structure among populations of the same species of *Otolemur*.

The small-eared greater galago (*Otolemur garnettii*) is the second largest galago species with a mean adult body weight ca. 770 g [Olsen and Nash, 2002]. This species is endemic to eastern Africa where it occurs in forest and woodland. There are currently four recognized subspecies [Grubb et al., 2003; Harcourt and Perkin, 2013; De Jong and Butynski, 2018; De Jong et al., in press]: Kikuyu small-eared galago (*O. g. kikuyuensis*) in the Kenya Highlands east of the Eastern Rift Valley (e.g. Nairobi, Ngong Hills, Aberdare Range, Mount Kenya, Mathews Range); Pangani small-eared galago (*O. g. panganiensis*) in extreme central southern Kenya (Loita Hills, Tavetta), southwards through eastern Tanzania (Mount Kilimanjaro, Lake Manyara, Eastern Arc Mountains, Southern Highlands) to extreme northern Mozambique (Ruvuma River); Zanzibar small-eared galago (*O. g. garnettii*) is restricted to three Tanzanian islands in the Indian Ocean (Zanzibar, Pemba, Mafia); white-tailed small-eared galago (*O. g. lasiotis*) in extreme northeastern Tanzania (Tanga) northwards along the coastal plain of Kenya to the Juba River in southern Somalia (Fig. 1) [De Jong and Butynski 2009, 2018; Harcourt and Perkin, 2013; De Jong et al., in press].

[[[Figure 1. here

Kessler et al. [2015] compared temporal and frequency parameters of what they referred to as *O. garnettii* ‘bark calls’ from eight captive individuals. They described barks as individual call units that are emitted either singly or as part of a longer, more complex, call series. They equated the call series to ‘trailing calls’ [Bearder et al., 1995] and ‘long calls’ [Becker et al., 2003]. The long calls identified by Becker et al. [2003] do not, however, follow the same structure as the trailing calls described by Bearder et al. [1995]. In addition, the calls analysed by Kessler et al. [2015] were triggered by disturbance. They may not be equivalent to the call

units that comprise spontaneous trailing calls, but rather are more similar to alarm calls. They found what they described as ‘moderate’ evidence for individual difference but no evidence for difference based on sex.

Here we provide an overview of the vocal repertoire of wild populations of *O. garnettii* and compare alarm calls and trailing calls (both referred to as ‘loud calls’ in many publications) of *O. g. panganiensis* and *O. g. kikuyuensis*.

Methods

Study Sites

We recorded vocalisations of *O. g. panganiensis* at two sites in northern Tanzania one encompassing narrow strips of riverine woodland and the other a banana plantation, and vocalisations of *O. g. kikuyuensis* from four sites in central Kenya, which include mid montane forest and riverine or non-riverine woodland (Fig.1; Table 1). These sites represent a range of habitats and populations.

[[[[Table 1 here.

Recordings of Vocalisations

We recorded calls using one of two methods. First, in Kwa Kuchinja and Aberdare NP, we placed one or three autonomous recording units (ARUs, Wildlife Acoustics SM2) at sites where *O. garnettii* were known to occur. We placed the ARUs >800 m apart and set them to record continuously from 10 min before sunset until 10 min after sunrise. Recordings were made in stereo at a sampling frequency of 44.1 kHz and 16 bits and saved in wav format. At Mweka, Meru FR, Meru NP and Masinga Reservoir, we manually recorded calls using a Marantz PMD660 recorder and Sennheiser ME67 (Mweka) or ME66 (all other sites) microphone, with a sampling frequency of 44.1 kHz.

Acoustic Analyses

Using ARUs, we collected 12 hours and 20 minutes of audio data during one night in Aberdare NP, and 169 hours of audio data across 16 nights in Kwa Kuchinja. We used Syrinx software (J. Burt, Seattle, WA, USA) to visually identify and extract calls. Any recording that looked like a potential *O. garnettii* call was confirmed by ear. We then categorised

vocalisations based on temporal parameters, repetition rate, and frequency and amplitude modulations, and matched them where possible to the equivalent call in previous studies. We calculated the call rate as the number of calls/hour.

For structural analysis, we concentrated on the two most common call types; ‘trailing calls’ (most likely advertisement calls) and ‘clustered squawk’ (most likely an alarm call, possibly the same as the ‘loud calls’ or ‘barks’ of Becker et al. [2003]). We used Avisoft SASlab pro version 5.2 (Avisoft Bioacoustics, Berlin, Germany) to measure seven temporal and spectral parameters of a smaller sample of good quality trailing calls and clustered squawks. Trailing calls (Fig. 2) start with a rather flat unit (or note) followed by a number of frequency modulated units and increases in level towards the end of the call. Clustered squawks (Fig. 2) start with the loudest unit and subsequent units have a lower level. For each call we measured: (a) call duration from the start of the first unit to the end of the last unit (sec); (b) unit duration for all units in a call (sec); (c) interunit duration between all units in a call (sec); (d) number of units in a call; (e) peak harmonic (the harmonic with the highest amplitude); (f) minimum and maximum peak frequencies of the fundamental harmonic (Hz), measured from the unit within a call with the lowest peak, and the unit with the highest peak frequency in their fundamental harmonic, respectively. We compared these call parameters between *O. g. kikuyuensis* and *O. g. panganiensis*.

[[[Fig. 2 here.

We conducted all statistical analyses in R [R Core Team 2013]. For each call we calculated mean unit duration, mean interunit duration, and mode peak harmonic. Peak harmonic reflects the harmonic that is most likely to contain the frequency with the highest energy. Since the number of harmonics is not a continuous variable we used the mode. Subsequently, we tested all parameters for normality using the Shapiro-Wilks’ Test. None of the variables for trailing calls were normally distributed (all p values <0.003). For clustered squawks, only mean unit duration, mean interunit duration, and minimum peak frequency of the fundamental frequency were normally distributed (all other p values <0.006).

Call parameters were compared using the nrmv package available at <http://CRAN.R-project.org/package=nrmv>. This package performs nonparametric multivariate analysis of variance (MANOVA) tests based on the inference methodology described by Bathke et al.

[2008]. It allows multiple analysis of variance-like nonparametric tests in situations where the classical, parametric multiple analysis of variance is not applicable (e.g. small or unequal sample sizes, no normal distribution) [Ellis et al. 2017]. Nonparametric ‘relative effects’ are provided as effect estimators. ‘Relative effects’ measure the probability that a value obtained from one experimental group is larger than a value randomly chosen from the whole dataset. In addition to these global hypothesis tests, the package provides a more detailed comparison of which variables or factor levels contribute to any significant differences.

Results

Vocal Repertoire

The vocal repertoire recorded at Kwa Kuchinja and Aberdare NP, along with temporal measures and the likely equivalent calls from previous studies, is described in Table 2. The audio files for each call type are presented on www.wildsolutions.nl/garnettii. Spectral measures and samples sizes of the high-quality calls analysed are provided in Table 3. The two most frequently recorded call types are the trailing call and clustered squawk; both are given throughout the night (Fig. 3). Trailing calls start to be produced after 19:00 h and continue until shortly after 06:00 h. Trailing calls elicited a responding trailing call from at least one other individual in 47.6% of cases. There is little variation in the temporal distribution of trailing calls, although they increase slightly during 04:00-06:00 h. There are few clustered squawks before 19:00 h or after 06:00 h, whereas their occurrence increases slightly during 00:00-05:00 h.

[[[Table 2 here

It is not possible to determine the number of individuals recorded by this study. However, based on overlapping calls, distance among callers, and knowledge of the population at the study sites, we conservatively estimate a minimum of three individuals at Aberdare NP, nine at Kwa Kuchinja, two at Mweka and Meru NP, three at Meru FR, and one at Masinga Reservoir.

[[[Table 3 and fig. 3 here.

Subspecific Differences

We analysed a total of 31 clustered squawks and 258 trailing calls for subspecific differences. Due to differences in sampling effort and methods, we had a greater number of *O. g. panganiensis* calls (clustered squawk: Kwa Kuchinja N=20, Mweka N=2; trailing call: Kwa Kuchinja N= 218; Mweka N=4) than *O. g. kikuyuensis* calls (clustered squawk: Aberdare NP N=5, Meru FR N=3, Masinga N=1; trailing call: Aberdare NP N=18, Meru FR N=12, Meru NP N=6).

Trailing calls differed significantly between the two subspecies (Fig. 2); ANOVA-type test value $F_{3.96,490.56} = 21.61$, $p < 0.001$. Relative effects (Table 4) show that for the variables interunit duration, minimum fundamental peak, and mode peak harmonic, the probability that a randomly chosen call from *O. g. kikuyuensis* exhibited a higher value than a randomly chosen call from the full data set was 83%, 79% and 61%, respectively. The probability that a randomly chosen trailing call from *O. g. panganiensis* exhibited a higher value than a randomly chosen call from the full data set was 74% for number of units and 68% for call duration. Posthoc tests for individual variables showed that subspecies differed significantly in: number of units ($F_{1,123.98} = 37.57$; $p < 0.001$), interunit duration ($F_{1,123.98} = 51.95$; $p < 0.001$), call duration ($F_{1,123.98} = 18.43$; $p < 0.001$), mode peak harmonic ($F_{1,123.98} = 6.58$; $p = 0.01$), and minimum peak frequency of the fundamental harmonic ($F_{1,123.98} = 53.28$; $p < 0.001$; Fig. 4).

[[[Table 4 here.

Figure 4 here.

Clustered squawks also differed between the two subspecies (Fig.2); ANOVA-type test value $F_{3.50,81.21} = 3.145$, $p = 0.023$. Relative effects (Table 4) show that for the variables mode peak harmonic and interunit duration the probability that a randomly chosen clustered squawk from *O. g. kikuyuensis* exhibited a higher value than a randomly chosen clustered squawk from the full data set was 77% and 61%, respectively. The probability that a randomly chosen clustered squawk from *O. g. panganiensis* exhibited a higher value than a randomly chosen clustered squawk from the full data set was 74% for number of units, 72% for call duration, and 81% for maximum peak frequency of the fundamental harmonic. Posthoc tests for individual variables show that subspecies differed significantly in number of units ($F_{1,23.17}$

=4.58; $p=0.04$), mode peak harmonic ($F_{1,23,17}=7.89$; $p=0.01$), and maximum peak frequency of the fundamental harmonic ($F_{1,23,17}=7.11$; $p=0.014$; Fig. 5).
[[[Fig 5 here.

Discussion

This study is the first to present an overview of the range of *O. garnettii* calls produced by wild populations in Tanzania and Kenya, as well as the temporal distribution of the two most common calls, clustered squawks and trailing calls. We show that *O. g. panganiensis* and *O. g. kikuyuensis* differ in the structure of these two calls, with trailing calls differing in more parameters than clustered squawks.

We describe 11 call types for *O. garnettii*. The currently described vocal repertoires of nocturnal primates ranges from two in Calabar angwantibo (*Arctocebus calabarensis*) [Charles-Dominique, 1977]) to 18 in northern lesser bushbaby (*Galago senegalensis*) [Zimmermann, 1985]. In contrast, for diurnal primates the size of the vocal repertoire ranges from six in the common woolly monkey (*Lagothrix lagotricha*) [Casamitjana, 2002] to 38 in bonobos (*Pan paniscus*) [Bermejo and Omedes, 1999].

The graded call system [Marler, 1977] of *O. garnettii* makes it challenging to separate some call types. Our estimate of 11 call types is, therefore, conservative. Some call types were recorded more frequently than others. This is partly a reflection of the recording method as ARUs do not record low volume calls unless the caller is close to the recorder. This implies that close contact calls are underrepresented relative to the louder advertisement and alarm calls. Since we did not record at least three call types previously reported from captive *O. garnettii* at close proximity [Becker et al. 2003], or in response to artificial stimuli [Kessler et al., 2015], it is likely that the vocal repertoire presented here can be expanded by at least three call types. Nonetheless, among nocturnal primates, only *G. senegalensis*, with 18 call types, is known to have more call types than *O. garnettii*.

Vocal repertoire size has been linked to social complexity in primates [McComb and Semple 2005]. Like many other nocturnal primates, *O. garnettii* has a dispersed social system where females, but not males, have over-lapping home ranges [Nash and Harcourt, 1986]. These authors state that adults usually sleep alone whereas at Kwa Kuchinja adults frequently sleep

in pairs or even groups of up to four (C. Bettridge, unpublished data). It is possible that variation in the social organization of *O. garnettii* is influenced by habitat, and specifically the availability of suitable vegetation [Nash and Harcourt, 1986]. The relatively large vocal repertoire of *O. garnettii* suggests that individuals which sleep or forage alone remain in regular contact with conspecifics [Charles-Dominique, 1978].

There is little temporal variation in the occurrence of trailing calls and clustered squawks; both are produced throughout the night between 19:00 h and 06:00 h. Loud calls are common across a wide range of primate taxa and are species-specific acoustic signals that carry over long distances [Gautier and Gautier, 1977; Hohmann and Fruth, 1995; Wich and Nunn, 2002; Delgado, 2006]. They are thought to advertise the location of the caller and to provide other information such as caller identity, sex or quality. Most nocturnal prosimians use loud, repeated, distinct calls for distant communication [Zimmermann, 1990].

It is most likely that the trailing call of *O. garnettii* functions as an advertisement call. Additional evidence for this is that 50% of all trailing calls triggered a responding trailing call from conspecifics (Table 2). The slight increase in the occurrence of trailing calls during 04.00–06.00 h may be related to maintaining social cohesiveness prior to reaching sleeping sites, as in the golden brown mouse lemur (*Microcebus ravelobensis*) [Braune et al., 2005]. The function of clustered squawks is less clear, although they have been described as distress or alarm calls [Honest, 1996; Becker et al., 2003].

In this study we identified variation in the acoustic structure of the trailing call and clustered squawk between *O. g. panganiensis* and *O. g. kikuyuensis*. The trailing call of *O. g. panganiensis* is of lower frequency than that of *O. g. kikuyuensis*. In contrast, the clustered squawk of *O. g. panganiensis* is of higher frequency compared to *O. g. kikuyuensis*.

It has been proposed that in closed habitats vocalisations will be longer with a lower repetition rate and lower frequencies as an adaptation to the habitat-specific transmission requirements (see Ey and Fischer [2009] for a review). *Otolemur garnettii* lives in forests, forest-agriculture mosaics, and woodlands. The majority of *O. g. panganiensis* calls used in this study were obtained from a narrow strip of riverine woodland that is surrounded by savanna. The vegetation in this habitat is less dense than in the forests where the *O. g. kikuyuensis* calls were recorded. It seems unlikely, therefore, that the lower frequency of the

trailing call of *O. g. panganiensis* is related to habitat structure. Furthermore, if habitat-specific transmission requirements select for spectral structure of calls, then we would expect both the trailing call and the clustered squawk to differ in the same direction in the two subspecies. We conclude, therefore, that the differences observed in this study are likely the result of lack of gene-flow (isolation), rather than an adaptation to local sound transmission characteristics.

Otolemur g. panganiensis appears to be separated from *O. g. kikuyuensis* by a geographical gap of ca. 45 km between Nairobi and Lake Magadi that includes the Taru Desert and Eastern Rift Valley (Fig. 1). The floor of the Eastern Rift Valley at Lake Magadi is low (600 m asl), wide (>32 km), dry (mean annual rainfall ca. 400 mm), hot (mean maximum temperature ca. 35 °C [Bennun and Njoroge, 1999]), and lacks perennial rivers [Butynski and De Jong, 2018]. This gap lies within the Northern *Acacia-Commiphora* Bushlands and Thickets Ecoregion [Olsen et al., 2001]. The habitats in this arid region are unsuitable for *O. garnettii* and, serve as a barrier between *O. g. panganiensis* and *O. g. kikuyuensis*. The shortest distance between the two *O. g. panganiensis* recording sites and the four *O. g. kikuyuensis* recording sites is ca. 280 km (between Mweka and Masinga Reservoir). This area is largely comprised of the Taru Desert which also lies within the Northern *Acacia-Commiphora* Bushlands and Thickets Ecoregion. The Taru Desert includes the Amboseli Basin and Tsavo West NP, extends westward towards Lake Magadi and northward to the Athi River near Nairobi (Fig. 1). The Taru Desert is ca. 1100–1500 m asl, has a mean annual rainfall of ca. 300 mm, and holds no perennial rivers [Butynski and De Jong, 2018], and, therefore, does not have habitats suitable for *O. garnettii*. The Taru Desert separates three subspecies of *O. garnettii*: *panganiensis*, *kikuyuensis*, *lasiotis*.

The Eastern Rift Valley and Taru Desert are also barriers for other forest-dependent primates; Mount Kilimanjaro guereza (*Colobus caudatus*), Mount Kenya guereza (*Colobus guereza kikuyuensis*), Mau Forest guereza (*Colobus guereza matschiei*) [Butynski and De Jong, 2018]; Zanzibar Sykes's monkey (*Cercopithecus mitis albogularis*), Kolb's monkey (*Cercopithecus mitis kolbi*). In addition, they represent the northern limit for miombo silver galago (*Otolemur crassicaudatus monteiri*) [De Jong and Butynski 2018].

Identifying the degree of intraspecific variation in calls is important if differences in calls are to be used in taxonomic assessments or to determine which calls and call parameters relate to

transmission requirements in their habitat rather than to genetic divergence. Comparing our results for the trailing call of *O. g. panganiensis* and *O. g. kikuyuensis* with those obtained by Masters [1991] for *O. g. lasiotis* and large-eared greater galago *Otolemur crassicaudatus*, shows some interesting patterns. Parameters for the trailing call for the three subspecies of *O. garnettii* differ from those of *O. crassicaudatus* — particularly the temporal features. This is congruent with the current taxonomy (Table 5).

The trailing call also differs among the three subspecies of *O. garnettii*; call length and number of call units are more similar between *O. g. kikuyuensis* and *O. g. lasiotis* than between *O. g. kikuyuensis* and *O. g. panganiensis* or between *O. g. lasiotis* and *O. g. panganiensis* (Table 5). Some of these spectral differences might be attributed to different measurement techniques used by Masters (1991) and this study. We cannot, however, exclude the possibility that the differences are genuine, and could support further taxonomic revision.

Using ARUs to record the calls means we do not know the number of calling individuals, nor the sex and age of the individuals in our sample. This is counteracted, to some extent, by the use of multiple ARUs at Kwa Kuchinja, by placing ARUs in different locations on different nights, and by the inclusion of calls from multiple, distant populations of each subspecies. Evidence from Kessler et al. [2015] suggests that *O. garnettii* calls cannot be discriminated by sex, so the differences in our results are unlikely to be due to sex differences in our sample. Age is also unlikely to be a major factor, as in mammals, alarm calls in particular undergo minor modification during the individual's development. Alarm calls in young individuals appear almost adult-like on first expression, suggesting that there is little role for vocal learning in the production of alarm calls [Blumstein and Munos, 2005; Hollén and Radford, 2009].

Most of the calls used in this study were recorded over a short time. Although some recordings were obtained from both subspecies at the same time of year, we do not have enough information about the study populations to know how they might relate to mating season. More data on the behavioural context of calls, perhaps obtained using playbacks to elicit responses, would shed light on the function both of the trailing call and clustered squawks.

Conclusion

The trailing call and clustered squawk (both long-distance calls) of *O. garnettii*, differ between *O. g. panganiensis* and *O. g. kikuyuensis*. This difference appears to be the result of a lack of gene-flow across habitats in southern Kenya that are unsuitable for *O. garnettii*. These differences confirm putative classification based on morphological traits of these populations into subspecies [Grubb et al., 2003; Harcourt and Perkin, 2013]. ARUs are useful tools for behavioural and taxonomic studies of galagos and other nocturnal primates.

Acknowledgements

Data in Tanzania were collected by students at the College of African Wildlife Management, Mweka. We are grateful to the College of African Wildlife Management for permission to record calls on their property, and logistical support in the field, and to TAWIRI for granting permission to record galago calls (Reference: AD.C.12 Vol.11.68.2014). We thank Amie Wheeldon for providing recordings from the Aberdare NP, and Kenya Wildlife Service and NACOSTI for permission to record calls at the four sites in Kenya (NACOSTI/P/18/5151/21938). Andrew Wolfenden and Roisin Stanbrook kindly helped set-up and monitor the ARUs. Finally, we are grateful to the two anonymous reviewers whose thoughtful comments greatly improved the manuscript.

Statement of Ethics

The authors have no ethical conflicts to disclose.

Disclosure Statement

The authors have no conflicts of interest to declare.

Author contributions

CB and SdK formulated the original idea and designed the study. CB, SdK, TB & YDJ collected acoustic recordings in the field. SPK and SdK conducted acoustic analysis. CB and SPK carried out statistical analysis. CB took the lead in writing the manuscript. All authors provided critical feedback and contributed to the writing of the manuscript.

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615

Table headings and figure legends

Table 1. The six sites at which recordings of the loud calls of small-eared greater galago (*Otolemur garnettii*) were obtained.

Table 2. Vocal repertoire of small-eared greater galago (*Otolemur garnettii*) showing temporal descriptive measures and equivalent calls from previous studies. Missing values are due to insufficient quality of recording.

Table 3: Frequency parameters for seven small-eared greater galago (*Otolemur garnettii*) calls.

Table 4. Relative effects of subspecies on acoustic parameters of small-eared greater galago (*Otolemur garnettii*) trailing calls and clustered squawks based on a nonparametric multivariate analysis of variance [Bathke et al. 2008]. Values range between 0-1 and indicate the chance that a randomly chosen call from a subspecies exhibits a higher value than a randomly chosen call from the full data set. Durations were measured in seconds; and frequencies in Hz.

Table 5: Species and population differences in trailing call parameters for large-eared greater galago (*Otolemur crassicaudatus*), white-tailed small-eared galago (*Otolemur garnettii lasiotis*), Pangani small-eared galago (*O. g. panganiensis*), and Kikuyu small-eared galago (*O. g. kikuyuensis*). The parameters include: N = total of number of calls included in analysis, LF(Hz) = mean lowest frequency in the fundamental harmonic, PF= the mean frequency with the highest amplitude in the call. Data for *O. crassicaudatus* and *O. g. lasiotis* from Masters [1991]).

Fig. 1: Geographic range of Pangani small-eared galago (*Otolemur garnettii panganiensis*) and Kikuyu small-eared galago (*Otolemur garnettii kikuyuensis*) in northern Tanzania and southern and central Kenya, with the recording locations depicted. Map based on De Jong & Butynski [2018] and De Jong et al. [in press].

Fig. 2: Cluster squawk (left panel) and a trailing call (right panel) of (top) Kikuyu small-eared galago (*Otolemur garnettii kikuyensis*) and (bottom) Pangani small-eared galago (*Otolemur garnettii panganiensis*). Within each panel, there is a spectrum (left), spectrogram (right), and waveform (top). The bottom right panel shows six temporal and spectral parameters measured for analyses: a) call duration (seconds); b) unit duration (seconds); c) inter-unit duration (seconds); d) harmonic with peak frequency (Hz); e) maximum peak frequency of fundamental harmonic (Hz); f) minimum peak frequency of fundamental harmonic (Hz). Parameter d) is also indicated on the top right panel to highlight difference between populations for that parameter. Spectrogram settings: FFT size = 1023; Hamming Window, temporal resolution = 8ms; frequency resolution = 8Hz.

Fig. 3: Temporal distribution of the trailing call and clustered squawk of the small-eared greater galago (*Otolemur garnettii*). The boxes represent the interquartile range (IQR); the heavy line is the median; and the whiskers represent 1.5xIQR; black dots are outliers.

Fig. 4: Trailing call of Pangani small-eared galago (*Otolemur garnettii panganiensis*) and Kikuyu small-eared galago (*Otolemur garnettii kikuyuensis*): a) call duration (seconds); b) number of units in call; c) mean inter-unit duration (seconds); d) peak harmonic number; e): minimum peak frequency of fundamental harmonic (Hz). The boxes represent the interquartile range (IQR); heavy line is the median; whiskers represent 1.5xIQR; open circles are outliers.

Fig. 5: Clustered squawks of Pangani small-eared galago (*Otolemur garnettii panganiensis*) and Kikuyu small-eared galago (*Otolemur garnettii kikuyuensis*): a) number of units in call; b) peak harmonic number; c) maximum peak frequency of the fundamental harmonic (Hz). The boxes represent the interquartile range (IQR); heavy line is the median; whiskers represent 1.5xIQR; open circles are outliers.